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Efficacy of diatomaceous earth formulations admixed with grain against populations of *Tribolium castaneum*

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Abstract

The efficacy of diatomaceous earth (DE) to control stored-products Coleoptera on stored grain was examined against several populations of the red flour beetle, *Tribolium castaneum* (Herbst). Four commercially available DE formulations were tested: INSECTO®, Perma-Guard™, Protect-It® and the diatomite used for the production of Dryacide®, each at six concentrations (100-1000 ppm). A great variation of efficacy was observed among the DE formulations tested. Protect-It at concentrations up to 400 ppm was found to be the most effective formulation to control red flour beetle populations. However, a concentration of 1000 ppm of Protect-It was necessary to control all adults of all populations. Most *T castaneum* populations, except one from Ivory Coast (Asm), were more than 90% controlled with INSECTO and Dryacide DE at 600 ppm. At this concentration, about 88% and 22% Asm adults died with INSECTO and Dryacide DE, respectively. Perma-Guard was the least efficient DE formulation to control *T castaneum* adults with three populations exhibiting some survival at 1000 ppm. Reduced susceptibility to DE was observed in two populations, Asm and Lab susceptible from Kansas (Lab-S). As neither population had been previously exposed to DE, it is suggested that red flour beetles may naturally vary in susceptibility to DE. In addition, it was found that some populations can be satisfactorily controlled with some DE formulations but not with others.

Keywords : Diatomaceous earth ; red flour beetle ; tolerance ; control ; wheat

1. INTRODUCTION

Synthetic insecticides have been used since the 1950s to control stored-products insects (Subramanyan and Hagstrum, 1995). However, because of increasing concern over worker exposure, reduced efficacy due to resistant insect populations, pesticide residues in human and animal food, and environment protection, alternative methods are needed to control stored-products insects. As an alternative to chemical control, diatomaceous earth (DE) has been increasingly used over the last decade and is recognized as an essential component of Integrated Pest Management (IPM) in stored-products (Korunic, 1999). DE is a soft rock that is the fossilised remains of unicellular algae called diatoms. Depending upon geological source, it is almost pure amorphous silicon dioxide, which is non-toxic to mammals (IARC, 1997). DE absorbs the insects' cuticular waxes which causes death from desiccation (Ebeling, 1971; Rigaux et al., 2001). In addition, DE abrades the cuticle (Ebeling, 1971).

The efficacy of DE to control stored-product pests were found to depend on different factors such as insect species, commodity, grain moisture, and temperature (Fields and Muir, 1995; Fields and Korunic, 2000). In addition to insect species, it was recently observed that insects of the same species but of a different origin, presented different levels of susceptibility to DE (Rigaux et al., 2001). DE insecticide efficacy varies with the geological origin of the DE mines (Snetsinger, 1988; Katz, 1991; McLaughlin, 1994), probably due to differences in physical and morphological properties of the diatoms (Korunic, 1998).

DEs are already registered in some countries to control stored-products pests. However, the available formulations are not equally effective against every pest species and there is a need to increase the efficacy of the formulations to decrease the dose of usage to an acceptable level for stored-food manufacturers. Ebeling (1971) felt that genetic resistance to DE was unlikely because of the physical mode of action. However, Korunic (1998) and Korunic and Ormesher (1999) discovered reduced susceptibility (1.3-2.2 fold) of *Tribolium castaneum* (Herbst), *Cryptolestes ferrugineus* (Stephens) and *Rhyzopertha dominica* (F.) adults exposed to DE for 5-7 generations, as compared with unselected laboratory strains. This suggested that insects might be able to develop physiological or behavioural resistance mechanisms to DE. In addition, it was recently observed that a population of *T. castaneum* that had never been in contact with DE, was naturally tolerant to the DE formulation Protect-It (Rigaux et al., 2001). Therefore, finding a formulation efficient against one population of one species does not permit extrapolation of the results to every population of the species examined. These results highlight the need to study the susceptibility of different populations of different species against DEs in order to develop formulations efficient against most insect species and populations.

Tribolium castaneum is one of the least susceptible stored-products insects to DE (see Fields and Muir, 1995 for a review; Korunic, 1994). Korunic and Fields (1995) observed that only 24 h were required to achieve 100% mortality of *C. ferrugineus* with 300 ppm of DE, while applying the same dosage under the same conditions to *T. castaneum,* required 21 days for 100% mortality. As the red flour beetle, *T. castaneum,* is one of the most common stored-products pests, a DE formulation able to control flour beetles should be able to control most insects occurring in stored food. In addition, it would be useful to know the concentrations of the various commercially available DE formulations needed to control the red flour beetle. In the present study, the susceptibility of several populations of the red flour beetle, *T. castaneum,* was investigated using four different commercially available DE formulations.

2. MATERIALS AND METHODS

2.1. Tribolium strains

Adults from seven strains of *T. castaneum* were tested for their susceptibility to DE (Table 1). The insects were reared with a mixture of whole wheat flour enriched with brewer's yeast (10/1-w/w) as rearing medium and kept in the dark at 30 ± 3°C and 65 ± 5% relative humidity (r.h.).

Table 1. *Populations of the red flour beetle, Tribolium castaneum, their origin, and mortality observed in the control (in % ±s.e.)*

Population	Origin and year of collection	Control mortality
Asm	Abidjan, Ivory Coast, 1989	1 ± 1
Japan	Japan	2 ± 1
Lab-S	Kansas, USA, 1960	2 ± 1
Pakistan	Pakistan, 1989	15 ± 3
Rio Desago	New Brunswick, Canada, 1976	10 ± 2
Sun Chong	British Columbia, Canada, 1976	5 ± 3
Thailand	Thailand	4 ± 2

2.2. DE formulations

Protect-It® is a mixture of freshwater DE (Hedley Technologies Inc., 2601 Mateson Bldvde. Suite 5, Mississauga, Ontario, L4W 5A8, Canada) with 10% silica aerogel to enhance insecticidal activity (Korunic and Fields, 1995). It is a beige-coloured DE containing approximately 83.7% amorphous silicone dioxide, 5.6% Al_2O_3, 2.3% Fe_2O_3, 0.9% CaO, 0.3% MgO, and 1.9% other oxides (e.g. TiO_3, P_2O_3), and 3-5% moisture content (m.c). The median particle size is 5µm. It contains significantly less than 1% (usually less than 0.3%) crystalline silica content (Z. Korunic, personal communication).

INSECTO® is a marine DE (Natural Insects products, Inc., North Eckhoff Street, Orange, CA, 92668, USA) with 10% food-grade bait. It is a buff-coloured powder that is 87% (w/w) amorphous silicon dioxide, with 2-4% m.c, and with a chemical composition of about 3% Al_2O_3, 1 % Fe_2O_3, and less than 1 % CaO, MgO, TiO_3, and P_2O_3. The median particle size is about 8.2 µm and particles range from 1 to 34 µm. Specific gravity is 0.23, pH is approximately 6.0 and it has a surface area of 10-20m^2/g (Subramanyam et al., 1994).

The freshwater DE Perma-Guard™ D-10 (Perma-Guard Inc., P.O. Box 25282, Albuquerque, NM, 87125, USA) is a fine white dust containing 93% SiO_2, 3% Al_2O_3, 1.3% Fe_2O_3, 1.1% CaO, 0.6% Na_2, 0.3% K_2O and 0.2% TiO_3 and a maximum 4.5% m.c. The median particle size is about 11.7 µm. Specific gravity is 0.2, pH is 7.6, surface area is 26-28 m^2/g, oil absorption (ASTM D281-84) is 116-120%, and water absorption is 150% (data from manufacturer).

The freshwater DE Dryacide® is a white dust that is 90% amorphous silicon dioxide, 2% m.c., with a mean particle size of 13-15 µm (McLaughlin, 1994). Dryacide® Silica gel is not useful for the treatment of stored products because its small particle size makes it difficult to use. It is also rated as a foreign substance when grain is graded. These disadvantages are overcome by the use of a patented process whereby DE is coated with silica aerogels (Hedges and Belford, 1985). Dryacide® is a gray dust that is 86% amorphous silica, 2% moisture, 8% clay, and 4% carbon from organic material in the original diatomite. The median particle size is about 11.1 µm (Aldryhim, 1990). As Dryacide® was not available at the start of the experiments, the diatomite used for production of Dryacide® was used (throughout the text, the term Dryacide DE is thus used in reference to this diatomite).

2.3. DE bioassay

For every DE formulation, six concentrations were tested: 100, 200, 400, 600, 800 and l000 ppm (ppm: part per million, mg of DE per kg of grain). DE was added to a jar containing 150 g of grains of wheat (13-14% m.c.) and then mixed by hand. For every concentration, three replications were performed. The 150 g of grains were then equally distributed among three glass jars. Fifty 1-3 months old unsexed adults were introduced into each jar. After 21 days, the grain was sieved and the numbers of live and dead insects were counted. Experiments were carried out in the dark at 30 ± 3°C and 65 ± 5% r.h. The insecticidal efficacy of every DE formulation was tested against every *T. castaneum* population. Untreated grains were used as a control.

2.4. Statistical analyses

Mortality was calculated by pooling the number of dead and alive beetles across every replication (Dagnelie, 1975), and the proportion of dead insects was calculated. The mortality observed in the treatment was corrected with the mortality in the control (Abbott, 1925). A bootstrap procedure was run with the R statistical software version 1.5.1 (Ihaka and Gentleman, 1996) to estimate the standard error of the corrected mortality. As probit/logit transformations did not give a straight line for most results, it was not possible to estimate the LC50 of the DE formulations against the different *T. castaneum* populations. Statistical comparisons were performed with Minitab version 13.20 (Minitab, 2001). Analysis of variance (ANOVA) and, when necessary, subsequent Tukey and Newman-Keuls multiple comparison tests were carried out. One-way ANOVA was first performed to compare the mortality observed in the controls. The efficacy of the four DE formulations and the different concentrations of DE were compared. For statistical purposes, analyses were performed without pooling the data. A three-way crossed ANOVA was run on arcsin transformed data after correcting for the mortality observed in the controls (Abbott, 1925).

3. RESULTS

The insecticidal efficacy of the DE formulations tested against different *T. castaneum* strains is presented in Tables 2-5. As expected, mortality was observed to increase with the DE concentration. However, a wide variation in susceptibility of *T. castaneum* strains to DE was found, with the variation being higher at lower concentrations. At the concentration of 100 ppm, some strains suffered less than 10% mortality, whereas a few strains had about 95% mortality.

Table 2. *Corrected mortality (in % ± s.e.) of Tribolium castaneum populations treated with the formulation of diatomaceous earth: Perma-Guard®*

Populations	**Perma-Guard® concentrations (in ppm)**					
	100	**200**	**400**	**600**	**800**	**1000**
Asm	3 ± 2	2 ± 2	8 ± 2	45 ± 4	59 ± 4	82 ± 3
Japan	15 ± 3	59 ± 4	93 ± 2	97 ± 1	99 ± 1	100
Lab-S	0 ± 2	7 ± 3	14 ± 3	45 ± 4	84 ± 3	90 ± 2
Pakistan	45 ± 5	80 ± 4	97 ± 2	100	100	100
Rio Desago	0 ± 4	24 ± 5	73 ± 4	71 ± 4	90 ± 3	87 ± 3
Sun Chong	17 ± 4	79 ± 4	96 ± 2	100	100	100
Thailand	24 ± 4	74 ± 4	98 ± 1	100	100	100

Table 3. *Corrected mortality (in % ± s.e.) of Tribolium castaneum populations treated with the formulation of diatomaceous earth: INSECTO®*

Populations	INSECTO® concentrations (in ppm)					
	100	200	400	600	800	1000
Asm	7 ± 2	16 ± 3	47 ± 4	89 ± 3	97 ± 2	97 ± 2
Japan	54 ± 4	90 ± 3	98 ± 1	100	100	100
Lab-S	23 ± 4	77 ± 3	99 ± 1	100	100	100
Pakistan	90 ± 3	98 ± 1	100	100	100	100
Rio Desago	61 ± 5	100	100	100	100	100
Sun Chong	94 ± 2	99 ± 1	100	100	100	100
Thailand	67 ± 4	95 ± 2	97 ± 2	99 ± 1	100	100

Table 4. *. Corrected mortality (in % ± s.e.) of Tribolium castaneum populations treated with the formulation of diatomaceous earth: Protect-It®*

Populations	Protect-It® concentrations (in ppm)					
	100	200	400	600	800	1000
Asm	1 ± 1	6 ± 2	76 ± 4	91 ± 2	98 ± 1	100
Japan	97 ± 1	98 ± 1	100	100	100	100
Lab-S	42 ± 4	89 ± 3	97 ± 1	100	98 ± 1	100
Pakistan	87 ± 3	100	100	100	100	100
Rio Desago	46 ± 5	92 ± 1	100	100	100	100
Sun Chong	70 ± 4	99 ± 1	100	100	100	100
Thailand	84 ± 3	100	100	100	100	100

Table 5. *Corrected mortality (in % ± s.e.) of Tribolium castaneum populations treated with diatomite, a diatomaceous earth used to produce Dryacide®*

Populations	Dryacide DE concentrations (in ppm)					
	100	200	400	600	800	1000
Asm	1 ± 1	2 ± 2	12 ± 3	23 ± 4	83 ± 3	97 ± 2
Japan	61 ± 4	87 ± 3	100	100	100	100
Lab-S	11 ± 2	5 ± 3	42 ± 4	96 ± 2	95 ± 2	97 ± 1
Pakistan	26 ± 5	80 ± 4	100	100	100	100
Rio Desago	1 ± 4	18 ± 5	97 ± 2	100	100	100
Sun Chong	6 ± 3	48 ± 4	99 ± 1	100	100	100
Thailand	15 ± 3	87 ± 3	100	100	100	100

Less variation was observed at higher concentrations. Mortality increased with concentration, but live beetles were still observed at 1000 ppm in some populations with some DE formulations (see Tables 2, 3 and 5).

Despite mortality in the controls varying between 1.0% (strain Asm) and 15.3% (strain Pakistan) (Table 1), the difference was not strongly significant (one-way ANOVA; $F_{6,14} = 2.91$, $P = 0.047$) and no results were rejected for the statistical analysis. Significant interactions were observed between populations and DE, populations and concentration and between DE and concentration (three-way crossed ANOVA, $F_{18,19} = 2.59$, $P = 0.002$; $F_{30,90} = 3.77$, $P < 0.001$ and $F_{15;90} = 3.20$, $P < 0.001$, respectively). Despite the significant interactions, mortality was significantly different for the seven populations examined, the doses and the DE formulations tested (three-way crossed ANOVA, $F_{6,336} = 197.36$, $P<0.001$; $F_{3,18} = 9.06$, $P = 0.001$; $F_{5,18} = 19.84$, $P<0.001$, respectively). To compare the efficacy of the four DE formulations, a Tukey test was conducted. Protect-It and INSECTO were not significantly different and Dryacide DE and Perma-Guard had a similar efficacy (Tukey test, $P > 0.05$).

We knew from previous works that different strains of the same species have different susceptibilities to DE (Rigaux et al., 2001), that DE concentration affects mortality and that DEs differ in their efficacies (Fields and Korunic, 2000), which was confirmed in this study. In addition, our study gives the opportunity to directly compare the susceptibility of different strains to different DE formulations. It would be interesting to know if one population, which is not satisfactorily controlled with one DE formulation, could be better controlled with another DE. To test this hypothesis, the mortalities observed at the concentrations of 100 and 200 ppm were compared for each of the four DE formulations and the seven *T. castaneum* populations. These concentrations were chosen because mortality did not achieve either 0% or 100% in most cases for the different strains (but see Table 2: Lab-S and Rio Desago 100 ppm, Table 3: Rio Desago 200 ppm and Table 4: Pakistan and Thailand 200 ppm). Mortalities of 100% were observed too frequently at the concentrations of 400 ppm and more. The results are presented in Table 6. Despite this, significant differences were not observed in every case. Asm was found to be the most tolerant population to the DE formulations tested. Lab-S was also one of the most tolerant populations, but was satisfactorily controlled with Protect-It at 200 ppm (Table 4). Three populations, Pakistan, Rio Desago and Thailand, presented a similar level of susceptibility to the four DE formulations examined, with Rio Desago being most tolerant. Of the two remaining populations, Sun Chong appeared to be highly susceptible to Perma-Guard and INSECTO but more tolerant to Dryacide DE and Protect-It. In contrast, beetles from the Japanese population were more susceptible to Dryacide DE and Protect-It and more tolerant to Perma-Guard and INSECTO.

Table 6. *Susceptibility of Tribolium castaneum populations to DE formulations. Comparisons were based upon the mortality observed at 100 and 200 ppm of DE*

DE susceptibility	Perma-Guard		INSECTO		Protect-It		Dryacide DE	
Most tolerant	Lab-S	a	Asm	a	Asm	a	Asm	a
	Asm	a	Lab-S	b	Sun Chong	b	Lab-S	a
	Rio Desago	a	Japan	c	Rio Desago	b	Rio Desago	a
	Japan	b	Rio Desago	c	Lab-S	b	Sun Chong	b
	Thailand	b	Thailand	c	Thailand	c	Thailand	c
	Pakistan	bc	Pakistan	c	Pakistan	c	Pakistan	cd
Least tolerant	Sun Chong	c	Sun Chong	c	Japan	c	Japan c	d

In the same column, populations followed by the same letter are not significantly different (Newman-Keuls test, P>0.05).

4. DISCUSSION

Different insects have different susceptibility to DE (Fields and Muir, 1995; Fields and Korunic, 2000). In addition, intraspecific differential susceptibility to DE was recently reported in the red flour beetle, *T. castaneum* (Rigaux et al., 2001) which was the first study to show that there is wide variation in susceptibility to DE within one species. These results may explain why different researchers obtain different results while using the same source of DE and the same insect species. As expected from Rigaux et al. (2001), we found that the different populations of *T. castaneum* examined here, displayed differential susceptibility to the DE formulations tested, with two populations (Asm and Lab-S) being tolerant to most DEs. In addition, we found that some populations, which are more tolerant to some DE formulations, can be satisfactorily controlled with other formulations (see Table 6).

Tests on Dryacide® for stored-product protection found that populations of the rice weevil, lesser grain borer, and red flour beetle showed 100% mortality with use of 1000 ppm at 65% r.h. and 20°C (Desmarchelier and Dines, 1987). In the present study, we found that, in the red flour beetle, at the same concentration and at 30°C and 65% r.h., 100% mortality was not reached in two strains of the seven examined, although, we were using an unrefined product.

DE is not widely used commercially, so it is unlikely that the difference between the strains is a response to selection pressure due to DE applications. Moreover, as DE has a physical mode of action, genetic resistance is unlikely to occur (Ebeling, 1971). However, it is possible that insects are able to develop physiological or behavioural resistance mechanisms to DE (Korunic, 1998; Korunic and Ormesher, 1999). Hence the tolerant strains had characteristics that made them pre-adapted to tolerate DE applications. We observed that Protect-It and INSECTO were the most efficient DE formulations. The high efficacy of Protect-It could be explained by the size of its particle, almost equal to INSECTO and smaller than the other two DE formulations tested. It was observed that the biological activity of the DE Celite 209 (used at 90% in INSECTO) increased significantly with reduced particle size. However, no correlation between particle size and activity was observed in the DE formulation "Macedonia" (Korunic, 1997). Particle size itself is thus not enough to explain the difference of efficacy among DE formulations. However, the small percentage of added silica gel significantly enhanced the efficacy of DE (Korunic and Fields, 1995). Also it is possible that the 10% of food-grade bait present in INSECTO may influence its efficacy against insects through internal desiccation due to feeding.

Among the stored-products pests, *Tribolium* flour beetles are one of the most resistant species to DE (Fields and Muir, 1995 for a review; Korunic, 1994, 1998). The control of the red flour beetle with DE may require the use of higher doses than those normally recommended. In the environment of stored products, the control of stored-products pests is balanced between the "zero insect tolerance" and the absence of pesticide residues in animal and human food. We thus need an insecticide which is free from residues and which is efficient against a broad spectrum of pests. DEs are now widely recognized as the solution to the second problem and are therefore an essential component of IPM in stored products (Korunic, 1999). The finding of a new DE formulation that is effective at low doses would help to store grain free of insects and chemical insecticide residues. The use of DE has been limited because of the necessity to use concentrations of 1000-3500 ppm to achieve a good efficacy, which significantly reduces grain bulk density and flowability, and creates visible dust residues (Subramanyam et al., 1994; Golob, 1997). Despite new DE formulations proposed to avoid this problem, as observed here, the high tolerance of flour beetle to DE has not yet been properly overcome. This could still limit the use of DE for stored-products protection.

Albeit increased temperature increases the efficacy of DE in most insects, species of the genus *Tribolium* (*T. castaneum* and *T. confusum* du Val) show greater tolerance at higher temperatures (Maceljski and Korunic, 1972; Aldryhim, 1990; Fields and Korunic, 2000). This phenomenon could be used to decrease the dosage of DE in grain silos necessary to achieve an acceptable control of flour beetles, i.e. using a combination of cooling by grain ventilation and DE treatment, resulting in the decrease of the LC95 against flour beetles. It has been found that DEs were more efficient when applied on wheat than when applied on other stored grain, e.g. milled rice, corn, oats and barley (Z. Korunic, personal communication). As our study was carried out with wheat, it is most probable that DE concentration may have to be increased in other types of grain to achieve a similar control to that in wheat.

Our results show that good control of the red flour beetle in stored wheat is not currently possible with the use of low DE concentrations. Despite variations observed between populations and DE formulations, on average we found that a control of about 95% of the adult population of the red flour beetle, *T. castaneum,* could be expected at treatments of 750 ppm of DE. However, ineffective control could be observed at this concentration depending on the DE formulation used (e.g. Dryacide DE or Perma-Guard, see results). It is therefore important to test each DE formulation against local strains of the red flour beetle to find out the acceptable concentration to control this species. A next step of the research would be the development of new DE formulations with an increased efficacy at lower concentrations in order to satisfy the expectations of the grain industry, manufacturers and consumers. This could be achieved by blending together DE formulations with good efficacy. With such a blend it would be possible to take advantage of several DE in one formulation, e.g. good desiccation power of one DE, great abrasive properties of another and small particle size of a third DE. With this kind of mixed formulation, the variation of efficacy observed against the different *T. castaneum* populations could be reduced.

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